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Previews

# STATus Report on Tetramers

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**STAT proteins bind DNA as dimers to regulate gene expression. Cooperative recruitment of pairs of dimers (tetramers) to adjacent DNA sites has also been documented. In this issue, Lin et al. (2012) examined tetramer function in vivo and showed that STAT5 tetramers function primarily as transcriptional activators.**

As recounted elsewhere in this issue (Stark and Darnell, 2012), signal transducers and activators of transcription (STAT) proteins were discovered 20 years ago as mediators of interferon (IFN)-stimulated gene induction. As the name implies, they were found to mediate transactivation of gene expression in IFN-stimulated cells, as a result of nuclear translocation triggered by JAK-dependent tyrosine phosphorylation. In the subsequent years, this paradigm for regulated induction of gene expression has been documented for many cytokine responses. Although highly related, specificity is conferred on the seven members of the vertebrate STAT family by virtue of their individual patterns of activation by particular cytokine receptors and to some extent by their individual DNA sequence recognition preferences. For instance, STAT6 displays the most divergent binding specificity, preferring an increased spaced palindrome relative to other STAT proteins, whereas STAT1-STAT2 dimers are recruited to DNA by an unrelated DNA binding component, IRF9. Other STAT proteins, such as STAT1, STAT3, STAT4, STAT5A, and STAT5B, share highly similar DNA binding specificities, complicating our understanding of underlying molecular elements of gene regulation by individual cytokines.

Gene activation is often accompanied by recruitment of activating proteins conferring epigenetic protein modifications, and STAT-dependent gene activation has been correlated with recruitment of histone acetyltransferases and the acetylation of histones H3 and H4. Phenotype analysis and gene expression data from STAT-deficient cells and mice support the notion that STAT proteins confer a high degree of specificity on cytokine response pathways through induction of specific patterns of gene expression. However, gene expression analysis of STAT-deficient cells or mice also revealed genes whose expression become derepressed in the absence of the corresponding STAT protein, suggesting that STAT proteins can confer both activating and repressing functions (Hennighausen and Robinson, 2008). Interpreting molecular mechanisms from STAT-deficient studies can be complicated. For instance, gene induction in the absence of a transcription factor does not prove that a given protein acts as a direct transcriptional repressor, because an equally likely scenario would be an indirect mechanism involving secondary absence of a STAT-induced repressor protein or other inhibitory mechanism. In addition, inappropriate neomorphic alterations occur when one STAT protein is allowed to fill the vacuum left by the absence of another. For instance, loss of STAT1 allows inappropriate activation of STAT3 under normally STAT1-activating conditions, and the opposite occurs in the absence of STAT3. Likewise, the ratio between STAT1 and STAT4 can dictate which gets activated after stimulation, modulating subsequent gene expression responses . Each of these mechanisms can contribute to increased expression of individual genes in the absence of a particular STAT protein, without implicating a directly repressive function for that STAT (Levy et al., 2011).

Nonetheless, there is accumulating evidence that STAT proteins may be direct transcriptional repressors under a minority of circumstances, in addition to generally being transcriptional activators. Perhaps the best examples of potential direct gene repression by STAT proteins come from analyses of STAT5. Activation of STAT5 in Th17 cells in response to interleukin-2 (IL-2) stimulation represses the activity of the *Il17* promoter, which is otherwise driven by activated STAT3 in response to IL-6 stimulation (Yang et al., 2011). Chromatin studies suggested that both STAT3 and STAT5 bound the same regulatory site on *Il17*, where STAT3 recruited histone acetyltransferase p300 causing increased H3K4 acetylation under IL-6 stimulation, whereas binding of STAT5 displaced STAT3 under IL-2 stimulation and instead recruited the HDAC-containing repressor complex NCoR2, resulting in reduced histone acetylation. Why STAT5 bound to *Il17* would recruit repressor complexes rather than the activators it recruits to many other promoters remains a conundrum.

A suggested answer to this puzzle came from studies of the regulation of the *Igk* locus in pre-B cells that proliferate in response to IL-7. Light chain gene rearrangement requires germline transcription, which is repressed by IL-7 in a STAT5-dependent manner and induced after IL-7 withdrawal (Johnson et al., 2008). Molecular analysis suggested two aspects of repression and activation of Ig-κ: STAT5 inhibition of E2 protein binding to an overlapping site in the *Igk* enhancer and increased chromatin modification with the methylated H3K27 repressive mark through STAT5-mediated recruitment of the histone methyltransferase Ezh2 (Mandal et al., 2011).

Repression of *Igk* transcription by STAT5 raises a similar conundrum: why is STAT5 a repressor at this site, recruiting Ezh2 instead of p300? A potential answer came from analysis of its binding site. Although STAT proteins are known to bind partially palindromic DNA sequences known as GAS elements, they can also bind cooperatively to two adjacent GAS

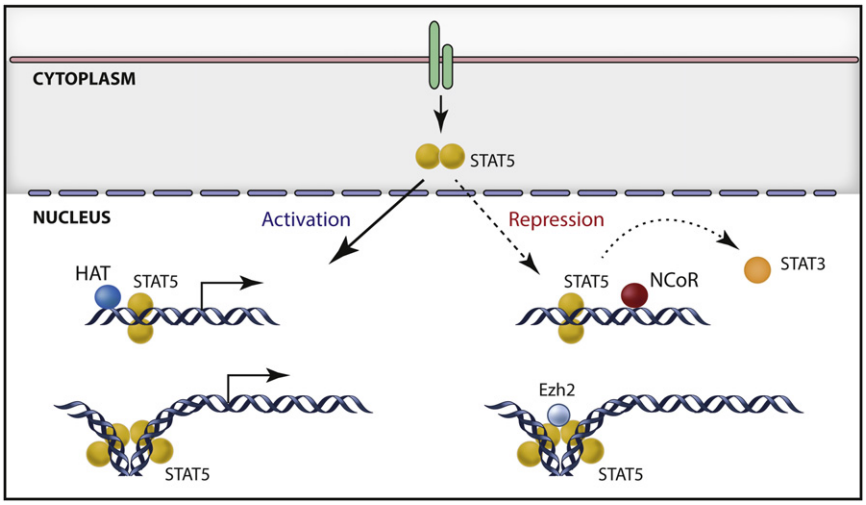


**Figure 1. Transcriptional Regulation by STAT5 Dimers and Tetramers**
Cytokine stimulation induces STAT5 dimerization through phosphotyrosine-SH2 domain interactions. STAT5 dimers are competent to bind DNA, and most STAT5 dimer binding sites were found close to promoters, where STAT5 served as an activator. A pair of STAT5 dimers can also bind to adjacent STAT binding sites in a cooperative manner, through interactions between the amino-terminal domains of the individual dimer pairs. Most tetrameric binding sites involve a high-affinity binding site adjacent to a lower-affinity site that is a multiple of half helical turns away, probably requiring DNA bending to allow dimer-dimer interaction. Tetramer binding sites tended to be located in intragenic and intronic regions, rather than near promoters, and drove the expression of genes involved in proliferation. Activation of gene expression by STAT5, either by monomers or dimers, probably involves recruitment of coactivator proteins with histone acetyltransferase (HAT) activity. STAT5 has also been implicated in gene repression, either by displacement of STAT3 binding and recruitment of the NCoR repression complex or by recruitment of the histone methyltransferase Ezh2. The rules governing the switch between recruitment of coactivators versus recruitment of corepressors by STAT5 remain to be defined, but the majority of direct STAT5-dependent action, whether by dimers or tetramers, appears to involve gene activation rather than repression.

elements separated by multiples of a half helical turn of DNA, through protein-protein interactions mediated by their amino-terminal domains (Vinkemeier et al., 1998). Cooperative binding to adjacent sites allows STAT recruitment to low-affinity sequences by increasing the duration of factor binding to otherwise weak sites, thereby expanding the repertoire of potential STAT-regulated genes. Repressive STAT5 binding to the *Igk* enhancer correlated with a dual GAS element binding STAT5 tetramers, suggesting that tetramer binding may provide a platform for Ezh2 recruitment instead of coactivators (Figure 1).

Although dimer versus tetramer discrimination between coactivator and corepressor recruitment is an attractive solution to the conundrum of dual STAT5 activities, this notion doesn't appear to explain all the data. Indeed, tetramer binding was initially described in the context of gene activation (Vinkemeier et al., 1998), which has been confirmed by subsequent studies. In addition, although genome-wide analysis of STAT5 binding in pre-B cells documented additional examples of a convergence of STAT5 and H3K27 methylation in pre-B cells, none of these additional sites were associated with induction of gene expression resulting from loss of activated STAT5 after IL-7 withdrawal (Mandal et al., 2011). Therefore, although STAT5-mediated recruitment of Ezh2 may result in modified chromatin, in most cases this epigenetic state is stable, even after loss of STAT5. Similarly, STAT5 repression of *Il17* gene expression, presumably because of displacement of STAT3 binding, correlates not with increased H3K27 methylation but rather with decreased acetylation (Yang et al., 2011).

In this issue of *Immunity*, a new study (Lin et al., 2012) of the physiological role of STAT5 tetramers in vivo has shed additional light on mechanisms and consequences of STAT5-dependent gene regulation. Lin et al. (2012) created knockin mice that express compromised versions of STAT5A and STAT5B that are unable to form tetramers, as a result of mutations in their amino-terminal interaction domains. Homozygous knockin mice were fertile and viable, unlike STAT5-null animals, but they displayed discrete alterations in T lymphocyte development and function, particularly in responses to IL-2. Although gene expression studies implicated both increases and decreases in IL-2-stimulated gene expression dependent on STAT5 tetramers, the majority of gene expression alterations in the mutant animals were loss of gene induction, indicative of tetramers functioning as activators. Moreover, correlations between genome-wide identification of STAT5 binding sites with regulation of gene expression documented numerous examples of STAT5 binding associated with gene induction and no examples of chromatin-bound STAT5 associated with gene repression. The simplest interpretation of these data would be that STAT5 tetramers directly induce gene expression and only indirectly regulate gene repression, presumably through the positive regulation of an intermediate repressor.

Interestingly, many tetramer-regulated genes were involved in IL-2-dependent cell proliferation and survival. This will probably come as no surprise to Moriggl and colleagues, who implicated STAT5 tetramers in cell proliferation in the context of constitutive STAT5 activation in leukemia (Moriggl et al., 2005). STAT5-null cells reconstituted with mutant proteins capable of dimerization but unable to form tetramers failed to support leukemogenesis, presumably because of defects in induction of gene expression required for proliferation.

There is, of course, a caveat to the tetramer-deficient studies of both Lin et al. (2012) and Moriggl et al. (2005). Although both groups documented that their mutant proteins fail to form tetramers and therefore ascribe their data to this deficit, it is possible that other protein-protein interactions are also disrupted by these amino acid changes. Although absence of tetramers is the most likely explanation of the data, it is prudent to entertain the possibility of other molecular explanations.

These studies leave us with a view of STAT5 proteins, whether bound to DNA as dimers or as tetramers, as being mainly transcriptional transactivators, not repressors. The mutant mice created by

Lin et al. (2012) will be a valuable resource to more thoroughly investigate both the physiological requirements and molecular mechanisms of STAT5 tetramer function. It will be particularly interesting to examine B cell differentiation, particularly light chain gene rearrangement, in the tetramer-deficient mutant animals, to better understand whether the Ezh2-dependent repressive function of STAT5 tetramers is a special case operating only on a few genes under restricted cell type-specific conditions. If so, understanding the molecular elements governing such specific repression will undoubtedly uncover further unexpected nuances of STAT function. Similarly, the regulation of IL-17 by the divergent action of STAT3 and STAT5 begs for a molecular explanation. Examining Th17 cell differentiation in STAT5 tetramer-deficient mice will undoubtedly be revealing, as will assessment of the development of proliferative disorders. If tetramer-deficient mice display resistance to leukemia as predicted by earlier studies, targeting disruption of amino-terminal interaction domains could be a novel therapeutic approach.

STAT proteins continue to surprise us, even after 20 years of investigation. Even nontranscriptional and extranuclear functions of STAT3 and STAT5 have been documented (Lee et al., 2012), which must also be taken into account when assessing STAT protein action. We can only imagine what the next 20 years of research will reveal.

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# Toll Signaling in Flies and Mammals: Two Sorts of MyD88

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**The mammalian MyD88 signaling molecule participates in Toll receptor signaling within the cytoplasm. In this issue of *Immunity*, Marek and Kagan (2012) report that *Drosophila* (d)MyD88 acts instead at the plasma membrane to sort the signaling adaptor Tube.**

The importance of Toll receptors in immunity was first recognized some 15 years ago in the fruit fly *Drosophila melanogaster*, where Toll plays a crucial role in the resistance to fungal and Gram-positive bacterial infections. These findings were then rapidly extended to mammals (Hoffmann, 2003). These transmembrane receptors relay information regarding the presence of infectious microorganisms to the cytosol through signaling transducers, which share with Toll-like receptors (TLRs) and the cytokine receptors of the interleukin-1 family a 150 amino acid domain known as the TIR (Toll-IL-1R) domain. This domain functions as a homotypic protein-protein interaction domain. Interestingly, studies in mammals have revealed that the four TIR signaling transducers in mammals belong to two functional categories: MyD88 (the prototypic member of the family) and TRIF behave as signaling adaptors, interacting with downstream signaling kinases and TRAF ubiquitin E3 ligases, and the two others, known as TIRAP and TRAM, function as sorting adaptors and recruit MyD88 and TRIF, respectively, to the plasma membrane and the endosome (Barton and Kagan, 2009). The sole TIR domain cytosolic adaptor in *Drosophila*, dMyD88, was believed to function as a signaling adaptor. However, Marek and Kagan (2012) now report that this molecule contains a phosphoinositide (PI) binding domain and functions as a sorting adaptor. These results open new perspectives in the field of Toll signaling and reveal that the sorting of transducing adaptors to particular membrane domains may represent an evolutionarily ancient property inherent to Toll signaling.